THE GENOTOXIC EFFECT OF LEAD AND ZINC ON COWPEA (*VIGNA UNGUICULATA*) AND MAIZE (*ZEA MAYS* LINN.)

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ABSTRACT

An investigation was carried out on the treatment effect of lead and zinc on the chromosomes of cowpea and maize. The seeds of cowpea and maize were placed in Petri dishes in three replicates and allowed to germinate for 5 days in different concentrations: 25mg/L, 50mg/L and 100mg/L of both lead and zinc nitrates respectively, while the control group had distilled water. The total chromosomal aberrations were examined. The mitotic index was calculated and the results were statistically evaluated by the analysis of variance at 5% significant level. The mitotic index decreased as the metal concentration increased ($P < 0.05$). The highest mitotic index values were 2.70 ± 0.83 and 3.40 ± 0.88 for the cowpea and maize untreated plants (control) respectively, while the least values observed were 0.20 ± 0.13 and 1.80 ± 0.70 for cowpea and maize treated with the 100 mg/L Zn respectively. The results showed the most frequent chromosomal anomalies induced by these heavy metals as stickiness, vagrants and bridges. Pb was found to be more toxic than Zn, as no germination was observed in both plants for the highest concentration of Pb tested. For Zn, there were no aberrations observed in cowpea at the highest concentration tested as well, while 22.22% aberrations were observed for maize. Pb induced greater aberrations having percentage abnormalities of 48.0%, 21.74% while Zn had 35.71%, 28.57% in maize and cowpea respectively for the 25mg/L concentration. As more abnormalities accumulate, gamete formation is affected and this lead to non-viable gametes, which considerably reduces plant fertility. The results from this study showed that Pb and Zn can induce genotoxicity in plants and these suggest potential health risk to human populations.

Key Words: Chromosomes, Aberrations, Genotoxicity, Heavy Metals, Mitotic Index.

INTRODUCTION

Cowpea (*Vigna unguiculata* (L) Walp) is a popular leguminous staple food in Nigeria (Adaji *et al.,* 2007) and constitutes an important source of dietary protein and secondary staple carbohydrate. Cowpea is grown mainly for its protein-rich grains and quality fodder for livestock. Islam *et al.* (2006) noted that cowpea is more tolerant to drought, infertile soils and acid stress than common beans. It is a semi-arid crop adaptable to a wide range of geographical and environmental conditions including poor soil and limited rainfall. Cowpea has been the subject of genetic research since the beginning of the 1900s. (IITA, 2010).

In Nigeria, maize (*Zea mays*) is the most important staple cereal after sorghum and millet and it has the widest geographical spread in terms of production and utilization among cereals (Omoloye, 2009). Maize or corn is a versatile cereal crop that is grown widely throughout the world in a range of agro-ecological environments. Maize is the most important cereal crop in sub-Saharan Africa (SSA) and an important staple food for more than 1.2 billion people in SSA and Latin America (IITA, 2010). Production and utilization of maize has increased recently in Nigeria as a result of the introduction of high yielding, drought-tolerant, early and extra-early maturing varieties (Omoloye, 2009).

Rapid industrialization had led to regional and global redistribution of metals with consequent environmental pollution. The role of environmental pollution on the production of various types of deleterious effects in diverse living systems has been well established. Heavy metals are the most hazardous pollutants as they are non-degradable (Cline and Reed, 1998).

Heavy metals according to Ademoroti (1996) are defined as metals with density greater than $5g/cm^3$. These according to him include transition metals and metals with higher atomic weights of groups III to V of the Periodic Table. Heavy metals become pollutants when the quantity present in living organism is greater than the tolerable quantity for good functioning of the

system. Metals get to plants from various sources like the earth's crust, soil erosion, mining, industrial discharge, urban runoff, sewage effluents, air pollution fallout and pest, weed or disease control agents. These heavy metals are passed through the food chain and are toxic to both plants and animals as a result of bio-accumulation and bio-magnification. Among heavy metals, lead and Zinc are the major contaminants found in soil, sediments, air and water. Lead can remain in the environment for 150-5000 years. Once in water, it enters the food chain and adversely affects the flora and fauna.

Heavy metals can be extremely toxic in animals because they damage nerves, liver, kidney and bones, and also block functional groups of vital enzymes (Wang, 2002). Increased level of lead in soil caused significant reduction in plant height, root-shoot ratio, dry weight, nodule per plant and chlorophyll content in *Vigna radiata* (Tomar *et al.,* 2000). According to Sharma and Dubey (2005) lead (Pb) is one of the prominent examples for anthropogenic environmental metal pollution that originates from various activities including mining and smelting of lead-ores, burning of coal, effluents from storage battery industries, automobile exhausts and additives in paints and gasoline. On Zinc-rich soils, only a limited number of plants have a chance of survival. That is why there is not much plant diversity near Zinc-disposing factories (Heyman, 1991).

According to Oladele *et al.* (2013) the highest Pb and Zn concentrations tested, induced 92.3% and 30% percentage chromosomal aberrations respectively in Bambara groundnut. Information relating to the effect of heavy metals deposited within a specified system as well as their genotoxic effect on living organisms is necessary for better understanding of the long-term effects of heavy metals. As a result, in this study, the treatment effect of lead and zinc on the chromosomes of cowpea and maize was investigated.

MATERIALS AND METHODS

Dry seeds of Cowpea (*Vigna unguiculata)* accessions*:* TVU 3788 and Maize (*Zea mays*) accessions: ACR.91SUWANI-SRC1 were collected from the International Institute of Tropical Agriculture (I.I.T.A.), Ibadan. The metal salts used were lead nitrate and zinc nitrate. The metal salts were purchased from Labio Scientific, Mushin, Lagos. Seeds were spread uniformly in Petri dishes lined with filter paper. The Petri dishes were divided into three replicates and the seeds were divided into two sets of metal treatment (Pb and Zn). Equal volumes of the different concentrations of lead and zinc nitrate solutions (25, 50 and 100 mg/L) respectively were administered while the control group had distilled water. The seeds were allowed to germinate within the Petri dishes and were treated with the different concentrations of each of the metals and distilled water respectively at a temperature of 25°C for 5 days. Growing root tips which were brittle, translucent and gently tapering were selected from the three plants grown in the effluents of different concentration and from the control. About 2-3 mm terminal root tips were cut off using a sharp blade and then placed on a clean glass slide and macerated with the aid of two dissecting needles and the remaining portion discarded. A drop of 1N Hydrochloric acid (HCL) was added to the root tip and left for 5 minutes; this softens the root tissue breaking up the middle lamellae. The excess acid was sucked up with a filter paper and the softened tissue was further macerated with dissecting needles so that the cells easily absorb the stain and spread adequately for microscopic observation. Then, a drop of lactic acetic orcein stain (2%) was placed on the macerated root tip and allowed to stand for 20 minutes for clearer viewing of the mitotic stages using the microscope (Michelle Frainer *et al.,* 2006). Each slide was covered with a cover slip and pressed down to allow the tissue spread out and also to allow the excess stain seep out at the edges of the cover slip. This was removed by placing the slide between the folds of the filter paper and maceration was done with the base of the dissecting set. All slides were examined under the light microscope with high power magnification (X 40 objective); then the good slides were preserved by sealing the edges of the cover slip with nail varnish to prevent the stain from evaporating. The photomicrographs of good slides were then taken under the oil immersion lens (X1000 objective) using a WILD M20 microscope with MPS 55 photoautomat attachment. The mitotic index was calculated according to Balog (1982) using the formula:

Mitotic Index (M/I)

$$= \frac{\text{Number of cells in mitosis per field}}{\text{Total Number of cells per field}} \times \frac{100}{1}$$

The results of the mitotic index were statistically evaluated by the Analysis of Variance at 5% significant level using Microcal Origin 5.0 software.

RESULTS AND DISCUSSION

The results for genotoxicity assay of the treated cowpea and maize are shown in Figures 1 and 2. For both plants, 25mg/L and 50mg/L of lead and zinc nitrates resulted in anaphase bridges, stickiness, vagrant, laggards, fragments, C-mitosis and scattered chromosomes. The highest treatment concentration of 100mg/L of Pb and Zn resulted in no germination by treated plants. Tables 1 and 2 also showed the total number of cells analyzed, mitotic index values and the number of chromosome aberrations observed in cowpea and maize treated with different concentrations of lead and zinc nitrates. There was a highly significant difference between both metal-treated set and the control set ($P < 0.05$).There was a decrease in mitotic index with increase in treatment concentrations. The mitotic index of the control experiment was found to be 2.70 and 3.40 for cowpea and maize respectively. As for the treatment, at concentrations of 25mg/L, 50mg/L and 100mg/L, the mitotic indices were found to be 2.30, 0.50,0.00, and 0.50,1.20,0.20 for Pb and Zn treatment respectively in cowpea (Table 1). Also, for maize, the mitotic indices were found to be 2.50, 0.30, 0.00, and 2.80, 2.50, 1.80 for Pb and Zn treatment respectively (Table 2). Thus, there was a negative correlation between the concentrations of the metal treatment and the mitotic indices obtained. Inhibition of mitotic index increased significantly with an increase in the concentrations of the metal solutions. Maize had higher mitotic indices compared to cowpea for most levels of Pb and Zn treatment.

The lack of germination observed with seeds treated with the highest concentrations can be traced to the toxic potentials of these metals. This observation is in agreement with the work of Islam *et al.* (2007) that heavy metals such as (Pb) strongly inhibits germination even at very low concentrations. Lead-induced inhibition of seed germination has been reported in *Hordeum vulgare, Oryza sativa* and Z. *mays* (Senger *et al.,* 2009). Inhibition of germination in cowpea and maize may result from the interference of these metals (Pb and Zn) with protease and amylase enzymes (Tomulescu *et al.*, 2004 and Senger *et al.*, 2009).

All the concentrations tested induced different types of chromosomal abnormalities and the frequency of abnormalities increased, in most cases, as the concentration of the metals increased. A concentration-dependent decrease in chromosomal abnormalities was observed for Pb and Zn treatment in maize while cowpea showed no tolerance, growth nor germination in the presence of Pb and Zn concentrations above 25mg/L and 50mg/L respectively. There was a linear correlation between the concentration of metals and the percentage of abnormalities in the treated sets especially for Pb. The total aberration (%) increased after 50 mg/L Pb exposure and a decrease was observed after 50 mg/L Zn exposure. This was probably due to increased metal concentration and subsequent detrimental effect especially for Pb. A concentration-dependent decrease in number of dividing cells was also observed. This suggests an interference with cell division.

These metals showed a strong depressive effect on the mitosis of both plants studied especially cowpea. Thus, there was a negative correlation between the concentrations of the metal treatment and the mitotic indices obtained. This suggests an inhibition of mitosis by these metals. Kumar and Tripathi (2007) performed similar studies on the genotoxic effects of different chemicals on different plant materials, they also observed inhibition of mitosis. According to Oladele *et al.* (2013), the induction of cytological disturbances in meiotic cells is of great concern, as it results in genetic abnormality. Chromosomal stickiness was observed in mostly higher concentrations of 100mg/L of the two metals used in the study. This abnormality has also been reported for several extracts and chemicals already investigated (Nwakanma *et al.*, 2009). According to Kumar and Rai (2007), scattering of the chromosomes as observed in this study may be due to disturbance of the spindle apparatus. From this study, both heavy metals can induce

chromosomal anomalies.

The statistical values ($P < 0.05$) obtained for Pb-treated sets are much lower than those for Zn-treated sets (Tables 1 and 2). Generally, Pb-treated sets had lower mitotic indices compared to Zn. The higher mitotic indices obtained by maize compared to cowpea for both Pb and Zn treatment showed maize as a good crop-model to study heavy metal stress tolerance especially at the genetic level due to its ability to withstand heavy metal stress conditions, compared to cowpea.

TABLE 1: Chromosome Aberrations in Cowpea Root Tips Cells Treated with Different Concentrations of Lead and Zinc Nitrate

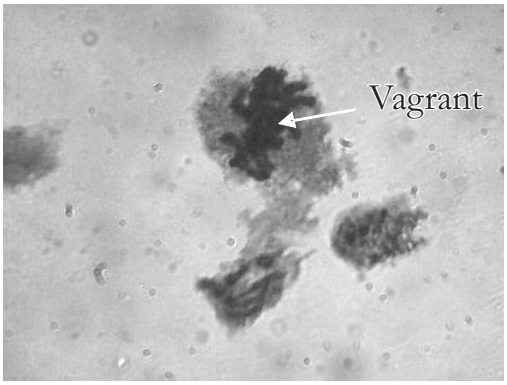
Treatment Concentrations	TCN	ND	ST	CM	BF	VG	LG	MA	TA (%)	MI	MI ± SEM	P-values
Control	1000	27($P_5M_{13}A_6T_3$)	0	0	0	0	0	0	-	2.70	2.70±0.83	0.0861**
25mg/L Pb	1000	23($P_1M_8A_{12}T_2$)	0	0	4	1	0	0	21.74	2.30	2.30±0.80	0.0032*
50mg/LPb	1000	5 ($P_0M_5A_0T_0$)	0	0	0	0	0	0	-	0.50	0.50±0.33	<0.0001*
100mg/L Pb	-	-	-	-	-	-	-	-	-	-	-	-
25mg/L Zn	1000	7 ($P_1M_6A_0T_0$)	0	0	1	1	0	0	28.57	0.50	1.40±0.33	<0.0001*
50mg/L Zn	1000	12($P_1M_6A_1T_4$)	0	3	0	0	1	0	33.33	1.20	1.20±0.52	<0.0001*
100mg/L Zn	1000	2($P_1M_1A_0T_0$)	0	0	0	0	0	0	-	0.20	0.20±0.13	<0.0001*

TCN, Total Cell Number, ND, Number of dividing cells, ST, Stickiness, CM ,C-mitosis, BF, Bridges fragment, VG, Vagrant, LG, Laggard, MA, Multipolar anaphase, TA, Total aberrations, P, Prophase, M, Metaphase, A, Anaphase, T, Telophase Data were expressed as mean ± SEM, MI, Mitotic index. * $P < 0.05$, Significantly different from control**, **** $P>0.05$, not significantly different from control

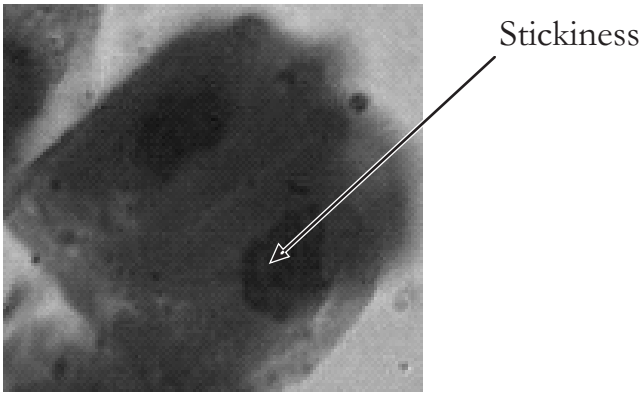
TABLE 2: Chromosome Aberrations in Maize Root Tips Cells Treated with Different Concentrations of Lead and Zinc Nitrate

Treatment Concentrations	TCN	ND	ST	CM	BF	VG	LG	MA	TA(%)	MI	MI ± SEM	P-values
Control	1000	34($P_2M_{15}A_5T_{12}$)	0	0	0	0	0	0	-	3.40	3.40±0.88	0.7376**
25mg/L Pb	1000	25($P_3M_9A_4T_9$)	4	0	2	5	0	1	48.00	2.50	2.50±0.82	0.0042*
50 mg/L Pb	1000	3($P_0M_1A_1T_1$)	0	0	0	1	0	0	33.33	0.30	0.30±0.21	<0.0001*
100mg/L Pb	-	-	-	-	-	-	-	-	-	-	-	-
25mg/L Zn	1000	28($P_7M_9A_4T_8$)	1	1	2	5	0	1	35.71	2.80	2.80±0.84	0.0050*
50mg/L Zn	1000	25($P_2M_{12}A_4T_7$)	4	0	4	2	0	0	40.00	2.50	2.50±0.83	0.0044*
100mg/L Zn	1000	18($P_3M_6A_1T_8$)	1	0	1	1	1	0	22.22	1.80	1.80±0.70	0.0031*

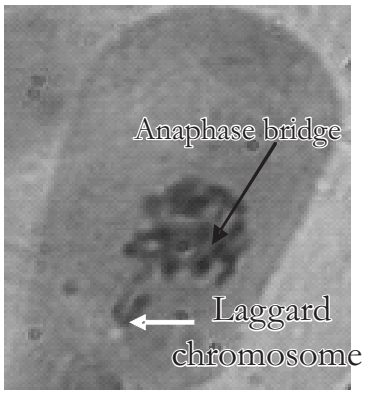
TCN, Total Cell Number, ND, Number of dividing cells, ST, Stickiness, CM ,C-mitosis, BF, Bridges fragment, VG, Vagrant, LG, Laggard, MA, Multipolar anaphase, TA, Total aberrations, P, Prophase, M, Metaphase, A, Anaphase, T, Telophase Data were expressed as mean ± SEM, MI, Mitotic index. * $P < 0.05$, Significantly different from control**, **** $P>0.05$, not significantly different from control



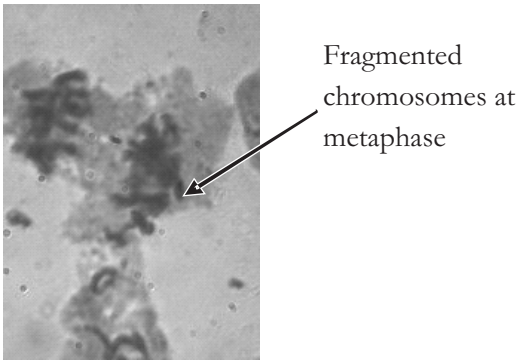
25mg/L lead nitrate x 40 (maize)



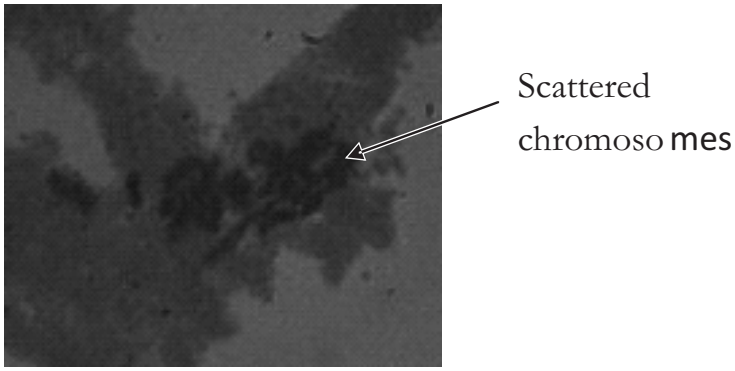
50mg/L Lead nitrate x 40 (maize)



25mg/L lead nitrate and 100mg/L zinc nitrate x40 (cowpea)



50mg/L zinc nitrate x 40 (cowpea)



50mg/L zinc nitrate x 40 (maize)

Figure 1: Chromosomal Aberrations Induced by Pb and Zn in Cowpea and Maize

A concentration-dependent decrease in number of dividing cells was also observed. This suggests an interference with cell division. There was also a negative correlation between the concentrations of the metal treatment and the mitotic indices obtained. This indicates an inhibition of mitosis by these metals. This is consistent with the observations of Kumar and Tripathi (2007) on different plant species. Chromosomal stickiness was observed in mostly higher concentrations of 100mg/L of the two metals used in the study. This abnormality has also been reported for several extracts and chemicals already investigated (Nwakanma *et al.*, 2009). According to Kumar and Rai (2007), scattering of the chromosomes as observed in this study may be due to disturbance of the spindle apparatus. From this study, both heavy metals can induce chromosomal anomalies.

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REFERENCES

Adaji, M.J., Olufaja, O.O. and Aliyu, L.2007. Effect of intra-row spacing and stand density on the growth and yield of cowpea (*Vigna unguculata* (L.) Walp). *In:* Olufaja, O.O., Omokore, D.F., Akpa, G.N and Sanni, S.A. (eds.). *Proceedings of the 41st Annual Conference of the Agricultural Society of Nigeria* (ASN) held at the Institute for agricultural Research, Samaru, Ahmadu Bello University, Zaria between 22nd and 26th October. 153 – 157pp.

Ademoroti, C.M.A. 1996. *Environmental Chemistry and Toxicology* Ibadan Foludex Press Ltd., London. 215pp.

Balog, C. 1982. The mitotic index in diploid and Triploid *Allium* roots. *Cytologia* 47: 689-697.

Cline,S.R. and Reed, B.E. 1998. Lead removal from soils via Bench Scale soils washing techniques. *Journal of Environment and Engineering* 121: 700-705.

Heyman, S.A. 1991. *Trace elements in Soil-plant-animal System*. Academic Press Incorporation, London. 417pp.

International Institute of Tropical Agriculture 2010. Maize : http://www.iita.org/crops/maize

Islam,.S, Cowmen. R., and Garner, J.O. 2006. Screening for tolerance of stress temperature during germination of twenty-five cowpea (*Vigna unguiculata* L. Walp) cultivars. *Journal of Food, Agriculture and Environment* 4(2): 189–191.

Kumar, G. and Rai, P. 2007. Genotoxic potential of mercury and cadmium in soybean. *Turkish Journal of Biological Sciences* 31: 13-15.

Kumar, .G and Tripathi, R. 2007. Lead induced cytotoxicity and mutagenicity in Grass Pea. *Turkish Journal of Biological Sciences* 32: 73-78.

Michelle-Frainer K, Antonio C.F, Thais .S, Solange, B.T. 2006. Effects of *Pterocaulon polystachyum* DC. (*Asteraceae)* on Onion (*Allium cepa*) Root-tip cells. *Genetics and Molecular Biology* 29: 539-542.

Nwakanma N.M.C, Odeigah P.G.C. and Oboh, B.O. 2009. Genotoxic Effects of *Gongronema latifolium* and *Vernonia amygdalina* using the Allium Test. In: *Book of Proceedings, 4th UNILAG Conference and Fair, Nigeria*, October 21-22, 2009, 81-90.

Oladele, E.O, Odeigah, P.G.C, and Taiwo, I.A . 2013. The Genotoxic effect of Lead and Zinc on Bambara groundnut (*Vigna subterranean*). *African Journal of Environmental Science and Technology* 7(1); 9-13

Omoloye, A.A. 2009. Field accumulation risks of heavy metals and uptake effects on the biology of *Sitophilus zeamais* (Coleoptera:Curculionide). *African Scientist* 10(2): 75–88.

Tomar, M., Kaur, I., Neelu, A.K. Bhatnagar. 2000. Effect of enhanced lead in soil on growth and development of *Vigna radiata* (L.) Wilezek. *Indian Journal of Plant Physiology* 5(1): 13-18.

Senger, R.S, Gautam, Garg, S.K., Senger, K., Chaudbary, R.2009. Lead stress effects on physiobiochemical activities of higher plants. *Rev. Environmental Contaminant. Toxicology* 196: 1-21.

Sharma P., and Dubey, R.S.2005. Lead toxicity in plants. *Brazilian Journal of plant physiology*. 17(1): 35-52.

Tomulescu, I.M., Radoviciu, E.M Merca, V.V, Tuduce, A.D. 2004. Effect of copper, zinc and their combinations on the germination capacity of two cereals. *Journal of Agricultural Science* 15: 15-17.

Verma, S. and Dubey, R.S. 2003. Lead toxicity induces lipid peroxidation and alters the activities of antioxidant enzymes in growing rice plants. *Plant Science* 164**:** 645-655.

Wang, W. X. 2002: Interaction of trace metals and different marine food chains. *Marine Ecology Progress Series* 243**:** 295-309.